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OESTRUS SYNCHRONISATION IN POSTPARTUM DAIRY COWS USING REPETITIVE PROSTAGLANDIN DOSES: COMPARISON BETWEEN D-CLOPROSTENOL AND DINOPROST

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This trial evaluated the reproductive performance in an early routine oestrus induction programme using two different $PGF_{2\alpha}$ preparations in dairy cattle. D-cloprostenol sodium (n = 192; Group A) or dinoprost (n = 187; Group B) was administered between days 35 and 42 post partum. Also, a group of non-treated cows (n = 135; Group C) was included as control. Pedometers were used to detect oestrus, and also secondary oestrous signs and vaginal mucus quality were assessed prior to artificial insemination (AI). When oestrus was not detected for 14 days after $PGF_{2\alpha}$ administration, the treatment was repeated, up to a maximum of three times. There were no differences between the study groups in oestrus detection (A = 73.48%, B = 73.01%, C = 79.26%; P = 0.428), good mucus quality (A = 96.45%, B = 91.30%, C = 93.45%; P = 0.203) and the presence of mounting lesions (A = 98.58, B = 94.93%, C = 98.13; P = 0.414). First-service pregnancy rates were 19.78%, 15.64% and 32.03% in Groups A, B and C, respectively (P = 0.003). There were no inter-group differences for the interval from parturition to first AI. However, a significantly shorter interval from parturition to conception (92.17 days, 99.45 days, 118.93 days; P = 0.002) and significantly less services per conception (2.12, 2.18, 2.66; P = 0.003) were observed in Groups A and B in comparison with Group C. The use of $PGF_{2\alpha}$ resulted in better fertility in a repetitive, routine postpartum programme, although no differences between D-cloprostenol and dinoprost were detected.

Key words: Oestrus cycle, prostaglandin F2 alpha, reproductive performance, bovine

The use of postpartum oestrus induction and synchronisation programmes has been widely studied to optimise the calving interval and thus increase the

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economic profitability of the herd. It is essential to restore postpartum reproductive status as soon as possible, which is dictated largely by the resumption of hormonal cycling and uterine involution, both of which are in turn regulated by the negative energy balance of this phase (Walsh et al., 2011). The difficulties inherent in postpartum reproductive efficiency are often aggravated by an unacceptably low rate of oestrus detection.

Prostaglandin (PG) treatments have been designed to improve the reproductive results after parturition by the use of, among others, single-dose administration, double-dose administration at 11- to 14-day intervals (Drillich et al., 2000; Répási et al., 2005; Stötzel et al., 2012), or combination of PGs with other active ingredients such as progesterone and GnRH (Pursley et al., 1995; Ribeiro et al., 2012). The currently available PGs differ largely in terms of their molecular composition (Pursley et al., 2012). The efficacy of commercial PGs is a matter of some debate, and depends to a large degree on the speed at which they are metabolised and on their affinity for myometrial and corpus luteum (CL) cell-membrane receptors (Re et al., 1994; Hirsbrunner et al., 1999).

The aim of this prospective study was to compare the effects on reproductive efficiency of a luteolytic dose of D-cloprostenol and dinoprost tromethamine, both of which were routinely administered at 35–42 days postpartum and repeated each 14 days in a commercial dairy cattle farm, as a strategy for improving the reproductive performance in dairy farms.

Materials and methods

Herd description, animal selection and treatment protocol

The present study was conducted in a commercial dairy farm with around 1200 lactating Holstein cows, located in Southern Spain (Jaen). The animals were housed in stalls with open-air and sheltered areas and fed a balanced diet, in accordance with the NRC requirements. All cows were milked twice daily at 6:00 and 17:00 h, with an average annual milk yield of 7500 kg per animal. A voluntary waiting period (VWP) of 35 days was established by selecting all cows at between 35 and 42 days post partum on the day when treatment commenced. Treatment was routinely administered every Monday; in cows not displaying oestrus, treatment was repeated 14 days later, up to a maximum of three times (Fig. 1). Cows were randomly assigned to two treatment groups: Group A (n = 192) received 0.15 mg of D-cloprostenol (Dalmazin, Fatro Iberica, Italy) and Group B (n = 187) received 25 mg of dinoprost tromethamine (Dinolytic, Pfizer, Germany). Mean parity was 2.29 ± 0.94 in Group A and 2.79 ± 1.07 in Group B. A total of 135 cows under standard reproductive management, without hormonal treatment for oestrus induction, were included as a control (Group C), with a mean parity of 2.55 ± 0.97. Body condition score was ≥ 2.5 in all animals studied (scale 0–5 units; Edmonson et al., 1989). For artificial insemination (AI), pedometers (Dairy Plan, Westfalia, Germany) were used to detect oestrus, which was recorded twice daily, during milking. Before AI, it was also recorded *(i)* whether cows showed typical oestrous signs on the back and hindquarters caused by scratches from other animals' hooves during mounting, and *(ii)* whether vaginal mucus, evaluated by vaginal examination, was adequate (abundant, clear, watery vaginal mucus was considered as good quality, and less abundant, thicker mucus was regarded as poor quality). Cows displaying purulent mucus consistent with endometritis were not inseminated. Pregnancy was diagnosed by rectal palpation between 45 and 60 days after insemination. Cows that returned to oestrus following AI or that were not pregnant at the time of diagnosis were not eligible to re-enter the study for subsequent AI.

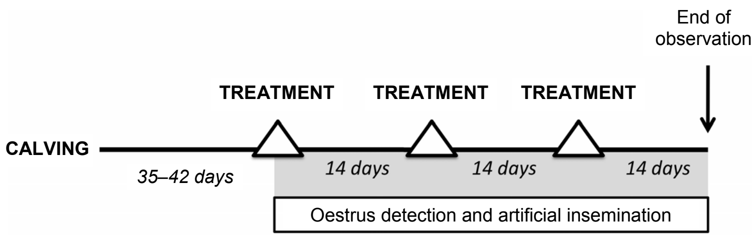

Fig. 1. $PGF_{2\alpha}$ schedule used to synchronise oestrus in postpartum dairy cows, indicating the times when oestrus detection and artificial insemination (AI) are carried out

Data management and statistical analysis

The following calculations were performed for both groups: oestrus detection rate (number of treated cows displaying oestrus), first-service pregnancy rate (number of cows becoming pregnant divided by the number of treated cows), first-service conception rate (number of cows diagnosed pregnant divided by the number of cows inseminated), percent of cows displaying oestrus with abundant and clear vaginal mucus, percent of cows displaying oestrus with typical oestrus-related signs on back and hindquarters, interval from parturition to first AI, interval from parturition to conception (days open) by 200 days, and services/gestation (by 200 days). Chi-square test was used to compare proportions, while intervals were compared by analysis of variance (ANOVA) and when differences were significant ($P \leq 0.05$), the Least Square Differences (LSD) test was performed. Results were expressed as mean ± SD. Survival curves were used to show the proportion of cows that did not demonstrate oestrus (and were not inseminated), and that did not conceive during the period of study. The statistical software package SPSS 11.0 for Windows was used to perform statistical analyses.

Results

No significant differences for oestrus detection rates were observed following the administration of D-cloprostenol or dinoprost, or in Group C (73.48%, 73.01% and 79.26%, respectively; P = 0.428). Figure 2 shows differences in the time elapsing to first AI (i.e. observed oestrus). Table 1 shows oestrus detection rates after the first, second and third prostaglandin administrations, where no inter-group differences were observed. In relation to the quality and quantity of cervical mucus at oestrus, largely abundant, clear and watery mucus was observed in 96.45% (136/141) of oestrous cows treated with D-cloprostenol, in 91.30% (126/138) of cows in the dinoprost tromethamine group, and in 93.45% (100/107) in the non-treated group; the differences were not significant (P = 0.203) (Table 1). The percentage of cows in which pedometer-detected oestrus was accompanied by mounting lesions did not show treatment-related variation (A = 98.58%, B = 94.93%, C = 98.13%; P = 0.414). A total of 13 cows (2.7%) were excluded from the treated groups (5 from Group A and 8 from Group B) due to vaginal discharge consistent with endometritis, while 7 cows were excluded from the non-treated group.

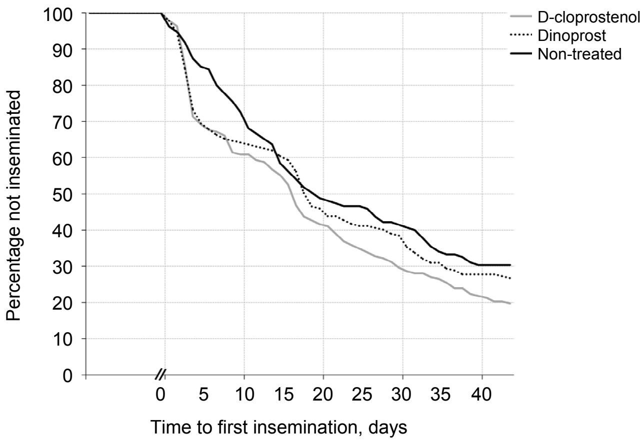

Fig. 2. Survival curves for days to first AI in dairy cows that were treated with D-cloprostenol (n = 192, grey line), dinoprost (n = 187, dashed line) or were left without treatment (n = 135, black line). There was no difference for the interval from parturition to first AI between groups. Day 0 indicates the beginning of the $PGF_{2\alpha}$ treatment or the voluntary waiting period (VWP) in the non-treated group (day 35 after parturition)

Overall first-service pregnancy rate was similar in Groups A and B (19.78% and 15.64%, respectively; Table 1), while Group C showed a significantly higher value (32.03%; P = 0.003). Cows showing oestrus after $PGF_{2\alpha}$ treatments had a significantly lower (P = 0.007) percentage of pregnancies (26.24% and 20.29% in

Table 1

Mean percentage of oestrus detection, presence of mounting lesions and vaginal mucus, and conception and pregnancy rates in postpartum dairy cows treated with different $PGF_{2\alpha}$ until oestrus induction

$PGF_{2\alpha}$ treatments for oestrus induction	Treatment groups			P value
	D-cloprostenol	Dinoprost	Non-treated	
At first treatment				
Detected oestrus rate (%, number)	40.10 (77/192)	40.64 (76/187)	–	0.917
First-service conception rate (%, number)	10.94 (21/192)	8.02 (15/187)	–	0.382
First-service pregnancy rate (%, number)	27.27 (21/77)	19.73 (15/76)	–	0.341
At second treatment				
Detected oestrus rate (%, number)	38.26 (44/115)	36.93 (41/111)	–	0.891
First-service conception rate (%, number)	10.43 (12/115)	8.18 (9/111)	–	0.648
First-service pregnancy rate (%, number)	27.27 (12/44)	21.95 (9/41)	–	0.621
At third treatment				
Detected oestrus rate (%, number)	31.25 (20/64)	30.0 (21/70)	–	1.00
First-service conception rate (%, number)	6.25 (4/64)	5.7 (4/70)	–	1.00
First-service pregnancy rate (%, number)	20.00 (4/20)	19.04 (4/21)	–	1.00
Overall				
Detected oestrus rate (%, number)	73.48 (141/192)	73.01 (138/187)	79.26 (107/135)	0.428
Adequate mucus (%, number)	96.45 (136/141)	91.30 (126/138)	93.45 (100/107)	0.203
Mounting lesions (%, number)	98.58 (139/141)	94.93 (131/138)	98.13 (105/107)	0.414
First-service pregnancy rate (%, number)	19.78 (37/187)^{a}	15.64 (28/179)^{a}	32.03 (41/128)^{b}	0.003
First-service conception rate (%, number)	26.24 (37/141)^{a}	20.29 (28/138)^{a}	38.32 (41/107)^{b}	0.007
Open days	92.17 ± 43.36^{a}	99.45 ± 57.54^{a}	118.93 ± 74.32^{b}	0.002
Days to first AI	52.24 ± 12.38	54.67 ± 39.67	62.21 ± 45.62	0.137
Number of AIs to pregnancy	2.12 ± 1.14^{a}	2.18 ± 1.32^{a}	2.66 ± 1.58^{b}	0.002

a, b: Different superscripts indicate significant differences between groups. AI: artificial insemination

Group A and Group B, respectively) than cows included in Group C (38.32%) during the period of the treatment, i.e. 80 (± 3) days after calving (Table 1). There were no inter-group differences for the interval from parturition to first AI (A = 52.24 ± 12.38; B = 54.67 ± 39.67; C = 62.21 ± 45.62; P = 0.137). In Groups A and B, cows needed fewer services to get pregnant than in Group C (2.12 ± 1.14; 2.18 ± 1.32; 2.66 ± 1.58, respectively; P = 0.0031). The number of open days was significantly lower in Groups A and B than in Group C (92.17 ± 43.36 days and 99.45 ± 57.54 days, versus 118.93 ± 74.32 days, respectively; P = 0.002).

Discussion

When selecting routine postpartum treatment schedules, priority must be given to the ease of use, scheduling convenience, clinical efficacy and cost effectiveness of the product. The final treatment choice will depend on the particular characteristics and purposes of the farm. Timed breeding systems are beneficial to the management of reproduction of dairy cows because they make it possible to schedule and control the insemination programme of lactating dairy cows. Repeated administration of $PGF_{2\alpha}$ is one of the most widely used treatments (Tenhagen et al., 2000). Therefore, oestrus synchronisation programmes should aim to control the follicular waves as well as the onset of oestrus and ovulation (Alnimer et al., 2009), in order to improve the oestrus detection and the pregnancy rates (Pankowski et al., 1995; Bissinoto and Santos, 2012). The results obtained in this study confirm that repetitive administration of D-cloprostenol achieves slightly better reproductive rates compared to dinoprost tromethamine, although the differences were not significant. In a similar study, Pursley et al. (2012) observed that differences between the two $PGF_{2\alpha}$ products were really apparent in first-parity cows.

After routine administration every 14 days, D-cloprostenol and dinoprost tromethamine proved equally effective in achieving synchronised oestrus induction in lactating dairy cows. Oestrus detection rates obtained with both $PGF_{2\alpha}$ products were higher than those reported in previous trials by Momcilovic et al. (1998) or Pursley et al. (2012) (52% and 46.7%, respectively), and a decline in oestrus detection rates after each successive dose of $PGF_{2\alpha}$ was observed. Animals failing to display oestrus after treatment should be further examined to ascertain the reason for the absence of oestrus.

Both studied PGs produced abundant, clear cervical mucus, perhaps due to an improved $PGF_{2\alpha}$-mediated follicular growth (Armstrong, 1981) and by the enhancement of oestradiol and LH secretion (Tsiligianni et al., 2001). Whether PGs have direct effects on follicular growth prior to the LH surge to initiate ovulation in livestock is unknown, but $PGF_{2\alpha}$ increases pituitary responsiveness to

GnRH to release LH in the postpartum cow (Weems et al., 2006). A very high proportion of cows detected in oestrus by pedometer showed mounting lesions. The results suggest that if this working protocol is adopted, the reproductive efficiency can be improved even in herds that use visual observation to detect oestrus. Although physical differences in cervical mucus quality are not believed to directly affect fertility (Tsiligianni et al., 2001), the results suggest that abundant, clear mucus may serve as an indicator of oestrus in farms using oestrus detection by observation, and may enable the timing of AI to be optimised. Similarly, $PGF_{2\alpha}$ administration leads to improved oestrus detection rates, since the number of mountings increases with the number of animals displaying oestrus, due to behavioural and pheromonal interactions. The increase in oestrous behaviour associated with more cows in oestrus could increase the number of oestrous cows detected with infrequent visual observation (Floyd et al., 2009).

At the onset of oestrus, 2.7% of the treated cows displayed whitish speckled cervical mucus consistent with genital infection. This rate is lower than the 15.6% reported by Drillich et al. (2000). According to Gautam et al. (2010), 25.3% of cows with postpartum endometritis (clinical or subclinical) during days 15 to 60 had persistence or recurrence of endometritis between days 61 and 150. This suggests that the repeated administration of $PGF_{2\alpha}$ outlined here is not only an efficient tool for oestrus induction and synchronisation, but may also have a therapeutic effect on mild (undetected) endometritis and thus enhance reproductive efficiency (Knutti et al., 2000; Tenhagen et al., 2001). The use of $PGF_{2\alpha}$ has also been described for treating subclinical endometritis (Janowski et al., 2011). However, the above-mentioned treatment sometimes does not affect the prevalence of subclinical endometritis, although it increases first-service pregnancy per AI (Galvão et al., 2009).

In both treated groups, oestrus distribution was highly heterogeneous, similarly as reported by Seguin et al. (1985), since follicular development was not controlled and maximum frequency of onset was recorded around 3 days after treatment, in agreement with the findings of LeBlanc et al. (1998). One factor limiting the efficacy of the repeat-dose $PGF_{2\alpha}$ protocol was the considerable variation of ovulation time, which rendered fixed-time AI impossible (Keister et al., 1999). A recurring doubt is whether or not the use of $PGF_{2\alpha}$ has a negative effect on fertility, due to either incomplete luteolysis or interference of the protocol with follicular dynamics. Lauderdale et al. (1974) and Drillich et al. (2000) deny the existence of any such negative effect. Minor improvements in pregnancy rates are proof enough to justify the financial benefits of using $PGF_{2\alpha}$ treatments (Plunkett et al., 1984). Treatment with additional $PGF_{2\alpha}$ increased the percentage of cows that have completed luteal regression but it did not have a detectable effect on pregnancies per AI (Brusveen et al., 2009).

In this study, it was observed that the first-service conception rate using D-cloprostenol was slightly higher than that obtained using dinoprost tromethamine, and similar as reported by others (Momcilovic et al., 1998; Keister et al., 1999; Drillich et al., 2000), although protocols differed between studies. In contrast, other studies concluded that both products were equally effective luteolysins and produced similar pregnancy outcomes in lactating dairy cows (Stevenson and Phatak, 2010). Cairoli et al. (2006) found no differences in conception rates between cloprostenol and dinoprost in cows inseminated at observed oestrus. In agreement with the findings of the present study, Salverson et al. (2002) reported that following administration of $PGF_{2\alpha}$ (dinoprost and racemic cloprostenol) in combination with MGA, the oestrus synchronisation and fertility results were similar for the two prostaglandins.

A number of studies report that the fertility rates obtained after routine administration of $PGF_{2\alpha}$ with no prior animal examination are higher than those obtained following administration of $PGF_{2\alpha}$ when a CL is detected by rectal palpation (Kristula et al., 1992; Heuwieser et al., 1997; Répási et al., 2014) or when high progesterone levels are measured (Heuwieser et al., 1997). In other studies, the accuracy of rectal palpation for the presence of a functional CL was 73.9%, which confirms the unreliability of rectal palpation alone to assess the functionality of the CL (Cairoli et al., 2006).

Cows included in Groups A and B required further 20 days to get pregnant in comparison with those in Group C, which represents a considerable financial drain. Knutti et al. (2000) observed that the interval between parturition and conception in cows with mild endometritis was shorter in non-cycling cows treated with $PGF_{2\alpha}$ than in cycling herdmates, but not significant differences were detected. This finding would seem to further highlight the advantages of the breeding programme mentioned here. No inter-group differences were observed in the interval between calving and first insemination. The interval was relatively short in both treated groups, thus allowing conception to take place sooner, and enabling cows with reproductive disorders to be treated earlier. In contrast to the findings of Revah et al. (1989), repeated administration of $PGF_{2\alpha}$ reduced the number of services required for the cow to become pregnant, thus underlining again the positive effect of $PGF_{2\alpha}$ on fertility.

In conclusion, the results obtained in this study demonstrate that routine postpartum administration of prostaglandins is a viable working protocol for commercial dairy farms with a high-quality oestrus detection system, enabling improved oestrus detection, concentration of AI and enhanced breeding efficiency, and significant differences between natural and synthetic $PGF_{2\alpha}$ were not detected in terms of the results achieved.

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